INVESTIGATIONS ON THE MANUFACTURING OF COCONUT WINE USING VARIOUS SUGAR SOURCES

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE (M.SC) IN BIOTECHNOLOGY

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DECLARATION

I hereby declare that the work which is being presented in this dissertation entitled "Investigations on the manufacturing of coconut wine using various sugar sources" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from 10.02.2025 to 31.05.2025 under the supervision of Dr. Prashant Shukla, Department of Biotechnology, Brainware University.

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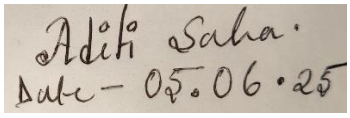

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CERTIFICATE OF INTERNSHIP COMPLETION

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Abbreviations:

Serial no	List of Abbreviations	Explanation
1.	CFU	Colony forming unit
2.	DNS	Di-nitro salicylic acid
3.	DPPH	2,2-diphenyl-1-picrylhydrazyl
4.	FTIR	Fourier Transform Infrared Spectroscopy
5.	RSA	Radical scavenging activity
6.	RSM	Response surface methodology
7.	TCW	Tender coconut water
8.	TPC	Total phenolic content
9.	TSS	Total soluble solids

ABSTRACT

This study focused on the development and detailed evaluation of coconut wine fermented using *Saccharomyces cerevisiae*, examining the effects of different sweeteners—sugar, date molasses, sugarcane jaggery, honey, and Nalen jaggery—as well as the addition of coconut pulp (Malai). Four groups of coconut wine formulations, with and without Malai and incorporating the various sweeteners, were fermented over 48 hours. The resulting wines were thoroughly analyzed for several physicochemical properties, including pH, alcohol content, residual reducing sugars, total phenolic content (TPC), and antioxidant activity measured by the DPPH assay. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) was used to detect chemical bond characteristics, and biochemical tests were carried out to evaluate the metabolic traits of the microbial strains involved. All wine samples exhibited pH values within the favourable range of 3.49 to 3.86. Among the sweeteners tested, sugarcane jaggery supported the highest levels of alcohol production—0.39% without Malai and up to 0.50–0.54% when Malai was included. In contrast, plain sugar resulted in the lowest alcohol yields. Generally, the presence of Malai improved alcohol output across most formulations. Wines made with sugarcane jaggery also showed the highest total phenolic content and antioxidant activity, suggesting potential health benefits. Biochemical tests of bacterial isolates revealed a range of enzyme activities (catalase, amylase, protease) and varied Gram staining patterns influenced by the type of carbon source used. FTIR analysis enabled molecular-level distinctions among different wine samples, variations influenced by the type of sweetener used and the inclusion of Malai. In summary, the type of sweetener used plays a critical role in influencing the fermentation efficiency and the functional quality of coconut wine. Sugarcane jaggery stood out as the most effective sweetener, enhancing both ethanol production and antioxidant capacity. The addition of Malai further supported alcohol fermentation, likely by offering supplementary nutrients or creating favourable conditions for yeast metabolism. These findings offer important guidance for optimizing coconut wine formulations to achieve specific quality and functional attributes.

Keywords: Coconut wine, *Cocos nucifera*, *Saccharomyces cerevisiae*, FTIR spectroscopy, pH, Total phenolic content (TPC), Antioxidant activity (DPPH assay), Alcohol concentration, Alcoholic beverage

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1.INTRODUCTION

Wine is a classic fermented alcoholic beverage created using yeast. Wines are undistilled drinks generally produced from fruit juices that are nutritious, mildly stimulating, and more flavourful than standard fruit juices. These fruits undergo fermentation, during which microbial processes play a crucial role in the final product's quality. Generally, wine contains an alcohol attention between 5 to 13 percentages. Wine possesses a flavour reminiscent of fresh fruit and can be stored and transported under conventional conditions. As an undistilled product, it retains most of the nutrients found in the original fruit juice. A standard wine comprises ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, and trace compounds that contribute to its flavour (Sreelekshmi *et al.* 2018). Fermented beverages and alcoholic drinks are culturally and socially embraced for various purposes, including consumption, entertainment, traditional customs, and religious ceremonies (Ezemba and Archibong 2017). Winemaking techniques are popular globally, spanning Europe, America, Australia, Asia, and recently Africa. For millennia, wine has served as a traditional medicinal drink and features prominently in various religious narratives. The production of wine is achieved primarily through anaerobic fermentation. Wine is believed to contribute to health, not only due to its nutritional benefits but also because it can substitute for unsafe, poor-quality, or unsatisfactory water supplies. It contains a significant amount of dietary fibre, which is helpful for individuals dealing with constipation. Additionally, it supports muscle growth in those who are thin or underweight (Ezemba and Archibong 2017).

The coconut, a fruit from the coconut win (Cocos nucifera), is piece of the Arecaceae kindred. It thrives in tropical areas typically located between 22° north and south of the equator, primarily along coastlines (Cuarto and Magsino 2017). The coconut palm is distributed across the western tropical lowlands but is capable of growing in any location with ample rainfall and warmth. While its exact origins remain unknown, it has been utilized for thousands of years, with Malaysia or Indonesia being the most probable early homes. One certainty is that humans have played a significant role in its dissemination throughout tropical regions, making it a common aspect of these areas (Bisht and Singh 2024).

Figure 1:Coconut wine (Tuba)

The term "coconut" can refer to the entire coconut palm, its seed, or the fruit itself. Botanically, it is considered a drupe rather than a true nut. Similar to other fruits, it consists of three layers: the exocarp, mesocarp, and endocarp. Inside the hard shell lies the seed, and when it germinates, the root of its embryo emerges through one of the shell's three "eyes" (Beveridge *et al.* 2022). Coconut water is the clear liquid present inside coconuts (the fruit of the coconut palm). This coconut water (also known as coconut liquid endosperm) is recognized as one of the most versatile natural products in the world. It occurs naturally, is extremely high in potassium, contains sodium chloride and carbohydrates, and is regarded as the preferred hydrating beverage in some regions In medical environments, coconut water can be utilized as an oral rehydration solution to restore fluids lost due to gastrointestinal issues, particularly in patients suffering from severe dehydration caused by diarrhoea. Meanwhile, an alcoholic beverage is characterized as a consumable liquid containing alcohol. There are three main categories of alcoholic beverages: fermented drinks, distilled spirits, and fortified wines. Fermented beverages are made from fruit juice or plant sap through natural fermentation processes. In India, various palms, including wild dates (Phoenix sylvestris), coconut palm (Cocos nucifera), and Palmyra (Borassus flabellifer), are commonly utilized for wine production. Wine is a type of alcoholic drink produced through the fermentation process. The yeast used in winemaking consumes sugar, converting it into ethanol, carbon dioxide, and heat (Zulaikhah 2019).

Coconut wine is a light pale beverage with a strong aroma and a sweet, refreshing flavour. It is a fermented drink produced from coconut water through the process of fermentation with the help of yeast (Jagadeesh *et al.* 2019). /. It is low in calories, almost fat-free, low in sugar, and contains a small amount of fibre which helps moderate absorption; additionally, it is rich in cytokines, or plant hormones, known for their anti-aging, anti-cancer, and antithrombotic properties in humans (Idise 2011). Coconut water can be utilized to create value-added products such as Nata-de-coco, therapeutic beverages, refreshing drinks (Jagadeesh *et al.* 2019).

Saccharomyces cerevisiae is a species within its genus that has a long-standing role in the fermentation and food processing sectors. Often called “wine yeast,” Saccharomyces cerevisiae is widely utilized in the creation of wine, bread, and beer. Despite its extensive application and familiarity, there have been no reports of negative effects on humans or the environment from Saccharomyces cerevisiae (Maicas 2020). The commercial use of the genus Saccharomyces cerevisiae originated in alcoholic beverages. This organism is preferred for alcohol production due to its high metabolic activity and its ability to tolerate elevated alcohol levels. The choice of species depends on the substrates used in the fermentation process as well as the alcohol requirements. Considering these points, the proposed study intends to create a quality fermented drink from coconut water using yeast (Varvaras and Kapolos 2020). The current study involved isolating yeasts from various fruit sources and optimizing and evaluating the beverage for different fermentation parameters with specified objectives.

2.Objectives:

To produce wine from coconut water (Cocos nucifera) using *Saccharomyces cerevisiae* for immediate consumption or preservation using refrigeration whenever the need, which will also be healthy and refreshing drink. The objective of this research is to evaluate the feasibility and optimize the process of producing wine from coconut (Cocos nucifera), utilizing coconut water and/or coconut sap as primary fermentation substrates. The study seeks to develop an efficient fermentation protocol by examining key variables such as sugar concentration, pH level, temperature, yeast strain selection, and fermentation duration to achieve optimal alcohol production. Emphasis is placed on employing Saccharomyces cerevisiae, a widely used wine yeast, to convert the natural sugars present in coconut into ethanol through anaerobic fermentation. The research involves systematic monitoring of the fermentation process, including measurements of Brix, pH, temperature, and microbial activity. Post-fermentation, the coconut wine is analyzed for its physicochemical properties such as alcohol content, total and volatile acidity, residual sugar, and pH, along with a sensory evaluation to assess flavor, aroma, and overall acceptability. The study also investigates the stability and shelf life of the product under various storage conditions. Furthermore, the potential economic and commercial viability of coconut wine is explored, considering the abundance and underutilization of coconuts in tropical regions, especially in coconut-producing countries. By transforming a readily available natural resource into a value-added alcoholic beverage, the research aims to provide an innovative and sustainable alternative in the fruit wine industry. The outcome of this study is expected to contribute to the diversification of fermented beverages, promote local agro-industrial development, and open new market opportunities for coconut-based products. Through the successful development and standardization of coconut wine production, the study hopes to enhance the commercial appeal of coconut while offering consumers a unique and naturally derived wine with tropical flavor characteristics.

3.Review of literature:

According to Giri *et al.* (2018), coconut water is a transparent, nutrient-dense liquid that is obtained from the endosperm of green coconuts. Its unique sugar, mineral, vitamin, enzyme, and hormone makeup accounts for its popular intake. Using Lactobacillus casei L4 to make fermented coconut water is beneficial. The beverage that is fermented with L. casei L4 for 48 hours includes antibacterial activity, antioxidants, vitamin B12, and important minerals. Fermented broth maintained the appropriate L. casei L4 levels, which are consistent with the daily recommended probiotic dose, even after being stored for 28 days at 4°C.

According to Sari *et al.* (2025), Indonesia's significant and growing coconut crop leads to a commensurate increase in the production of coconut water. In order to deal with this excess, bioethanol can be made by fermenting coconut water. Alcotec-48 Turbo Yeast is used to synthesize bioethanol using coconut water as a substrate. which leads to a low concentration of bioethanol, which distillation further increases. After hydrolysis, the initial glucose content of coconut water is 14% (v/v). This is used for fermentation with varying Alcotec-48 Turbo Yeast concentrations and varying fermentation times to produce higher bioethanol yields. On the sixth day of fermentation, 14g/L of Alcotec 48 Turbo Yeast produced the best fermentation outcome, producing a 37% (v/v) bioethanol content. Using 14g/L of Alcotec 48 Turbo Yeast on the sixth day of fermentation also produced the best distillation process outcome, with a 53% (v/v) bioethanol content. The best outcome of the distillation process was obtained with 14g/L of Alcotec 48 Turbo Yeast on the ninth day of fermentation, yielding a bioethanol content of 53.764%, while the best outcome of the fermentation process was obtained with 6g/L of Alcotec 48 Turbo Yeast on the second day of fermentation, yielding a bioethanol content of 19.7829%, according to the optimization results using Response Surface Methodology (RSM) with Minitab 17 application.

Jagadeesh *et al. (*2022) claims that Saccharomyces cerevisiae is one of the important yeasts that has been shown to be superior in fermentation technology. The clear liquid found inside young coconuts, known as coconut water, has also been used to make wine. Wine's TSS was continuously decreased until fermentation was finished. TSS decreased dramatically during the initial stage of fermentation, although it did so at a much slower rate until the fermentation was finished. It was shown that while the TSS level had no effect on the pH of coconut wine, the pH dropped as the fermentation time increased due to a rise in titrable acidity and alcohol. As fermentation went on, it was also observed that the titratable acidity increased, which was consistent with the pH drop. It was lowest at day six and highest at day twelve. Higher amounts of TSS during fermentation result in higher titratable acidity. The first day of fermentation immediately following inoculation sees a significant increase in the yeast count. After that, it tends to increase sharply until the third day, at which point the yeast count shows a fall until the fermentation is finished. Sucrose was added to coconut water as a source of sugar to create the coconut wine. The yeast isolate BRYI-1 yielded the highest results in terms of alcohol concentration and sensory qualities when compared to the reference strain, Saccharomyces cerevisiae (MTCC 171).

Kadere *et al.* (2004) highlight the coconut palm (*Cocos nucifera*) as a versatile tropical plant cultivated in about 90 countries. In Kenya’s coastal region, it is the most economically

significant palm species, with others such as *Borassus aethiopum*, *Hyphaene coriacea*, and *Phoenix reclinata* also present. Their study examined traditional coconut wine tapping and distillation practices, which remain widespread in the region. Tapping typically begins once trees reach a minimum age of ten years. The Kenyan method involves peeling the tip of the spathe's sheath and tying exposed spikelets with leaflets from the tree—a process distinct from Thai methods, where the sheath remains intact. Tapping and collection periods in Kenya can last one to three months, influenced by palm genotype, compared to about one month in Thailand. Effective tapping depends on the use of extremely sharp blades. Once sap is extracted, fermentation begins through the action of lactic and acetic acid bacteria, producing sour-tasting organic acids. Filtering is essential to remove debris, insects, and microbial by-products. The presence of acetic acid bacteria can also lead to the formation of polysaccharides like levan and dextran, potentially compromising wine clarity and quality. Traditional gourd containers are commonly used for sap collection, as they help maintain cool temperatures (25–30 °C), which slows fermentation and reduces frothing. While traditionally no preservatives were used, some producers now apply a mixture of sodium metabisulphite, sodium propionate, and sodium benzoate in a 10:1:1 ratio to extend shelf life. Distillation practices align with the "basin" method described by Nout for Nubian gin production, although significant differences exist in raw materials and residue management. In the Kenyan context, only coconut wine is used, and the resulting distillation residue is considered toxic to humans, animals, and plants—unlike in Nout's study, where residues were reused. Despite modern modifications, traditional techniques continue to shape sap identification, tapping, collection, storage, and distillation in coastal Kenya.

According to Lee *et al.* (2013), coconut water has emerged as a promising substrate for probiotic fermentation, offering dual benefits of hydration and health enhancement. Fermentation of coconut water using *Lactobacillus acidophilus* L10 and *Lactobacillus casei* L26 results in the successful proliferation and survival of probiotic cultures, with viable counts reaching approximately 10^8 CFU/ml after 2 days at 37 °C and sustaining 10^7–10^8 CFU/ml after 26 days at 4 °C. This highlights its potential as a functional probiotic beverage. While both strains exhibited similar growth and survival patterns, they differed in their metabolic interactions with coconut water components. Specifically, significant differences were observed in the metabolism of fructose, glucose, copper, phosphorus, and certain organic acids such as lactic, acetic, and malic acids. The study also revealed distinct volatile compound profiles between the two strains. *L. acidophilus* produced higher levels of methyl ketones and alcohols, such as 2-heptanone and 2-nonanol, while *L. casei* generated more esters and lactones, including diacetyl and ethyl 2-hydroxypropanoate, likely due to enhanced esterase activity. These volatiles may contribute to unique sensory characteristics, including buttery, fruity, and acidic notes. Despite notable acid production, substantial residual sugars remained, indicating suboptimal carbohydrate utilization. Importantly, fermentation did not significantly alter the mineral composition of coconut water, preserving its electrolyte balance and reinforcing its potential as a sports hydration beverage. The sodium content exceeded recommended levels for rehydration, suggesting enhanced effectiveness for fluid replacement.

These findings collectively underscore the feasibility of developing a novel, probiotic-rich coconut water beverage with both nutritional and sensory appeal.

Limbad *et al.* (2023), stated that the fermentation of coconut water kefir using different sugar sources, focusing on the associated microbial changes and biochemical developments over time. The growth of lactic acid bacteria was strongly influenced by both the duration of fermentation and the type of sugar used, with sucrose supporting significantly higher total viable counts than glucose. Yeast and acetic acid bacteria also exhibited growth, though less pronounced, with sucrose again emerging as the more effective carbon source. Shifts in organic acid composition were observed, notably an increase in lactic, pyruvic**,** and succinic acids and a decrease in malic acid, indicative of malolactic fermentation. Sucrose was metabolized more efficiently than glucose, aligning with increased microbial activity, higher titratable acidity, and a corresponding drop in pH. Amino acid analysis revealed a time-dependent rise in glutamic acid, contributing to improved flavour and potential health benefits. These patterns are consistent with those seen in other fermented foods like kimchi and milk kefir, where microbial metabolism drives similar transformations. The results underscore the kefir microbiota's preference for sucrose and point to strain-specific metabolic activities, such as GABA production. Overall, this research offers new perspectives on CWK fermentation by detailing how sugar type influences microbial dynamics, acid generation, and amino acid changes—areas that have been relatively unexplored in the context of coconut water fermentations.

According to Njoki *et al. (*2015), lactic acid bacteria can benefit human health as probiotics. They must survive in the gastro-intestinal tract. This study evaluated the probiotic activity of lactic acid bacteria strains obtained from fermented coconut wine. 15 Lactobacillus sp. strains were previously isolated from Mnazi. Several Lactobacillus strains isolated from Mnazi exhibited key probiotic characteristics, including resilience to acidic and bile conditions, antimicrobial effects, and survival under simulated gastrointestinal environments. Most strains remained viable at pH 2.5 with counts above 10^7 CFU/ml after 3 hours, although only eight were able to survive at pH 2. When exposed to 0.3% bile, survival was generally better at pH 2.5, with L. paracasei CB303 and CB4041 uniquely surviving and growing even at pH 2. Under simulated gut conditions with over 2% bile, five strains showed survival rates exceeding 50%. Antimicrobial testing revealed strain CB4041 had the strongest inhibition against both Gram-positive and Gram-negative bacteria, likely due to its production of bacteriocins and organic acids. Overall, these results suggest that Lactobacillus strains from Mnazi hold potential as effective probiotics and merit further investigation through in vivo studies.

According to Prasad and Sivamani *et al.* (2021), the pH, acidity, and ethanol concentration of water stored from Salalah and Sri Lanka coconut cultivars with and without dry yeast. This research explores how fermentation duration influences the pH, acidity, and ethanol levels in coconut water derived from Salalah and Sri Lanka cultivars, both with and without added dry yeast. Across a 28-day aerobic fermentation period, pH levels steadily declined while acidity and ethanol concentrations increased. Sri Lanka coconut water produced higher ethanol levels, reaching up to 2.935% (v/v) with yeast, compared to 2.092% (v/v) in Salalah. In contrast,

Salalah coconut water showed a greater increase in acidity, particularly with yeast, achieving 0.51 g/100 mL. Since a desirable fermented energy drink is characterized by higher acidity and lower ethanol content, Salalah coconut water appears more appropriate for such applications. Yeast-enhanced fermentation accentuated these effects across both cultivars. These findings support the potential of Salalah coconut water as a promising base for fermented functional beverages and contribute to ongoing research on natural plant-based fermentation processes.

According to Shiny *et al. (*2014), Tender coconut water (TCW) is a natural, nutrient-dense beverage valued for its electrolyte composition, which closely resembles human plasma. This makes it effective for hydration and suitable as a natural alternative to commercial sports drinks. It is rich in vitamins, amino acids, antioxidants, and enzymes. Fermentation—a widely used process that enhances food nutrition and digestibility—can be applied to TCW to increase its probiotic content. Probiotics, such as beneficial bacteria and yeasts, help maintain gut health by balancing intestinal flora, supporting immunity, and suppressing harmful microbes. Research suggests fermented foods are easier to digest, particularly for individuals with lactose intolerance. In the study, yeast was used to successfully ferment TCW, as sugar alone did not promote probiotic growth. The resulting beverage showed increased levels of protein, vitamin C, vitamin K, and key minerals like calcium and magnesium, while carbohydrate content decreased due to yeast activity. Sensory analysis showed the drink was well accepted, especially when combined with fruit juices. Additionally, fermented TCW demonstrated antimicrobial properties against several intestinal pathogens, highlighting its potential as a functional, gut-friendly health drink.

According to Nichanun *et al. (*2018), Coconut inflorescence sap, a sucrose-rich exudate from unopened coconut flowers, serves as a natural substrate for fermentation by native microorganisms, particularly *Saccharomyces cerevisiae*. These yeasts, often indigenous to specific regions, are favoured in wine production due to their adaptation to local environmental and fermentation conditions, which can enhance flavour and product quality. Studies have shown that over 50% of microbes isolated from fermenting coconut sap can produce over 9% ethanol, with *S. cerevisiae* dominating these communities. Selected strains from Thailand exhibited varied morphological and physiological characteristics, including carbon assimilation and temperature tolerance, with some producing over 67 g/L of ethanol. Five high-performing strains were used in coconut wine production, with NL010 yielding the highest alcohol content (10.3% v/v) and receiving the best sensory ratings. The use of autochthonous yeast strains not only preserves microbial biodiversity but also improves wine characteristics, supporting the growing preference for indigenous yeasts over commercial ones in modern fermentation practices.

According to Vargas *et al.* (2023), The coconut palm (*Cocos nucifera* L.), a vital perennial crop globally, plays a significant economic and cultural role, especially in countries like Cuba where it is predominantly cultivated in regions such as Baracoa, Guantánamo, and parts of Granma, Holguín, and Pinar del Río. While traditionally used for food, oil, and fibre, coconut's economic potential is currently underutilized due to declining dried coconut

production—from 21,298 tons in the 1980s to 7,499.9 tons by 2011—largely attributed to poor commercialization. This decline has spurred interest in diversifying coconut by-products, particularly through the production of fermented alcoholic beverages. The use of coconut water as a fermentation substrate presents challenges, including low initial sugar content (5.3 °Brix) and high pH (5.7), necessitating chaptalization and acidification to enhance yeast performance and product stability. Two fermented beverages were developed and analysed, revealing that both classified as sweet wines. Beverage 2 exhibited superior characteristics, including higher alcohol content (8.23%), acidity (2.58 g/L), and darker coloration, which contributed to better sensory acceptance (average score 5.89) compared to beverage 1 (score 3.76). These differences were statistically significant ($p \leq 0.05$), emphasizing the impact of optimized must conditions. Furthermore, low microbial counts ($<10^1$ CFU/mL) confirmed the efficacy of hygienic processing practices. Comparisons with other fruit wines indicate that the pH and acidity of the coconut-based products fall within acceptable ranges, although initial nutrient deficiencies in coconut water remain a limitation. Ultimately, the study highlights the potential of coconut water in producing stable, value-added beverages, provided that fermentation parameters are carefully controlled. This approach not only addresses declining coconut product yields but also opens new markets, increasing income opportunities for producers and enhancing the sustainability of coconut-based agro-industries.

4.Materials and Methodology:

4.1.Tools and Equipment's:

- glassware's,
- Analytical balance
- Tongs and gloves (for safe handling of hot crucibles)
- Laminar air flow
- BOD incubator
- Water bath
- Autoclave
- clean cheese cloth or fine sieve,
- Micro oven
- Colorimeter
- UV-vis Spectrophotometer
- pH meter
- Microscope

4.2.Chemicals –

- Nutrient Agar (for isolation of bacteria)
- MRS Media (Made using MRS broth and nutrient agar)
- potassium dichromate (dissolve 0.1g of potassium dichromate in 100 mL of distilled water).
- Distilled Water
- Concentrated H2SO4(to estimate alcohol)
- Ethanol (for standard curve)
- 1% 3,5-dinitrosalicylic acid (DNS)
- sodium potassium tartrate
- 2N sodium Hydroxide pellets (NaoH, for reducing sugar assay)
- Dextrose (For calibration curve)
- Centrifuge tubes and pipette
- Folin-Ciocalteu Reagent (1N): Commercially available, diluted to 1N with distilled water before use
- Sodium carbonate (Na_2CO_3)
- Gallic acid standard (for calibration curve)
- DPPH solution (0.1 mM in methanol)
- Crystal violet reagent (Primary stain)
- Gram's Iodine (a mordant)
- Ethanol (for decolorization)
- Counterstain safranin (for staining bacteria)
- 3% Hydrogen peroxide (for catalase activity)
- Starch(soluble)
- Peptone

- Beef extract
- Sodium chloride
- Skim milk powder (for protease activity)

4.3. Collection of Sample:

Coconut (cocos nucifera) fruit, sugar samples like sugar, sugarcane jaggery, Nalen jaggery, date molasses, Honey were obtained from local markets in Barasat, North 24 Paraganas, West Bengal, India.

4.4. Microorganisms used:

Yeast strain *saccharomyces cerevisiae* from department of Biotechnology, Brainware University, Barasat, Kolkata, west Bengal, India was used for fermentation process.

4.5. Preparation of coconut wine:

Fresh young coconut water was extracted and filtered through a clean cheesecloth or fine sieve to remove impurities. Four formulations were prepared as follows:

- **Set 1:** Coconut water mixed with sugar, date molasses, sugarcane jaggery, and nalen jaggery.
- **Set 2:** Coconut water mixed with sugar, honey, sugarcane jaggery, and nalen jaggery.
- **Set 3:** Coconut water and malai (coconut pulp) mixed with sugar, date molasses, sugarcane jaggery, and nalen jaggery.
- **Set 4:** Coconut water and malai mixed with sugar, honey, sugarcane jaggery, and nalen jaggery.

Figure 2: Coconut Wine by using 4 types of sugar samples

Each mixture was thoroughly stirred and transferred into sterilized fermentation containers using a funnel. Instant dry yeast was added to initiate fermentation. The containers were sealed with sterile cotton plugs and kept in a clean, dry area at room temperature for 48 hours. After fermentation, the cultures were stored at 4 °C for further analysis.

4.6. Bacterial Isolation and Probiotic Screening:

Small amount of wine sample inoculated in Nutrient agar media and incubated at 28 degrees Celsius. After the development of colonies, the bacteria will be pure cultured. And for further studies to know whether the bacteria are probiotic or not we've cultured them in MRS media as well, where the bacteria grew.

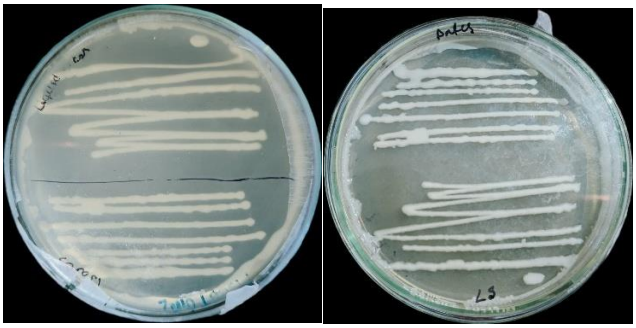


Figure 3: Streaking of a wine sample

4.7. Reducing sugar assay:

The DNS reagent was prepared by dissolving 1.6 g of NaOH (2N), 1% 3,5-dinitrosalicylic acid (DNS), and 25 g of sodium potassium tartrate in 100 ml of distilled water. To estimate reducing sugars, 3 ml of the DNS reagent was added to 1 ml of each wine sample along with 2 ml of distilled water in a test tube. The mixture was thoroughly shaken and heated in a boiling water bath at 100°C for 15 minutes. After cooling to room temperature, the absorbance was measured at 560 nm using a colorimeter. A standard curve was prepared using dextrose, and the reducing sugar content in the samples was determined based on this curve.

4.8. Test for pH:

The pH of the wine samples were examined using a digital pH meter (labrotonics) pre-calibrated with buffers of pH 4.0 and 7.0.

4.9. Estimation of Alcohol:

Alcohol was estimated using the potassium dichromate ($K_2cr_2o_7$) technique. In a 150-mL conical flask, dissolve 0.1g of potassium dichromate in 100 mL of distilled water. In a test tube, 1ml potassium dichromate and 1ml concentrated H2SO4 were mixed with 1ml of each wine sample and 4ml of distilled water to estimate alcohol. The absorbance was measured at 560 nm with a colorimeter. A standard curve was created using ethanol, and the alcohol concentration of the samples was calculated based on this curve.

5.0. Determination of total phenolic content:

The total phenolic content (TPC) in the wine samples was determined using the Folin-Ciocalteu method. Briefly, 1.0 ml of the wine sample was mixed with 1.0 ml of Folin-Ciocalteu reagent. After allowing the reaction to proceed for 3 minutes, 1.0 ml of saturated sodium carbonate (Na_2CO_3) solution was added, and the final volume was adjusted to 10 ml with distilled water. The mixture was then incubated in the dark for 30 minutes. Following incubation, the absorbance was measured at 760 nm against a reagent blank using a colorimeter. A standard curve was generated using gallic acid, and the total phenolic content of the samples was calculated accordingly.

5.1. Determination of antioxidant activity:

The radical scavenging activity (RSA) of the wine samples was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This method evaluates the antioxidant capacity of the sample based on its ability to reduce and decolorize the purple DPPH radical in a methanol solution. In this procedure, 5 ml of the wine sample was mixed with 5 ml of 0.06 mm DPPH solution prepared in methanol. The mixture was thoroughly mixed and incubated in the dark for 30 minutes. Following incubation, the absorbance was recorded at 520 nm. The percentage of DPPH radical scavenging activity was calculated using the appropriate formula.

Percentage RSA= [1−(Absorbance of control – Absorbance of sample)] ×100/

Absorbance of control

5.2. Fourier Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared Spectroscopy (FTIR) is a valuable technique for identifying the chemical bonds in solids, liquids, or gases. To prepare the samples for FTIR analysis using the Shimadzu Prestige 21 instrument, a drop of wine sample was mixed with potassium bromide (KBr) powder in a 1:100 weight ratio using an agate mortar. The resulting tablets were then analyzed, and the absorption spectra were recorded over a range of 400 to 4000 cm-1.

Figure 4: Fourier Transform Infrared Spectroscopy (FTIR)

5.3. Biochemical Analysis:

To evaluate the metabolic and enzymatic functions of microorganisms participating in coconut wine fermentation, a series of biochemical tests were performed. These included the catalase test, amylase test, protease test and Gram staining, among others, to determine enzyme activity and microbial traits relevant to the fermentation process.

5.3.1. Gram staining:

Gram staining is a laboratory technique used to classify bacterial species into two categories based on the characteristics of their cell walls: Gram-positive and Gram-negative. In coconut wine production, this method can be utilized to identify and track the microbial communities that play a role in the fermentation process.

During the fermentation of coconut wine (known as *tuba* or *toddy* in different regions), various microorganisms, including yeasts, lactic acid bacteria, and other bacteria, naturally thrive in the coconut sap. Monitoring these microbes is crucial because:

- **Yeast** (*Saccharomyces cerevisiae*) is responsible for fermenting sugars into alcohol, the main goal in coconut wine production.
- **Bacteria** can either enhance the wine's flavour, cause spoilage, or lead to contamination. Some lactic acid bacteria may spoil the wine, while beneficial ones contribute to flavour development.

Gram staining helps classify these microorganisms into two main groups:

- **Gram-positive bacteria**: These bacteria have a thick cell wall that retains the crystal violet stain. In coconut wine, lactic acid bacteria (like *Lactobacillus*) may play a role in fermentation or spoilage.
- **Gram-negative bacteria**: These bacteria have a thinner cell wall and an outer membrane, causing them to take up the red counterstain (safranin). Examples include spoilage organisms like *Pseudomonas*, which can produce off-flavours or unwanted by-products.

Procedure of Gram staining for coconut wine:

i. collecting a sample from the coconut sap or fermentation liquid.
ii. Smear a small amount onto a clean microscope slide and let it air dry, then heat-fix it by passing the slide through a flame.
iii. Flood the smear with crystal violet dye for 1 minute, then rinse off excess dye.
iv. Apply iodine solution for 1 minute to form an iodine-crystal violet complex, then rinse again.
v. Decolorize with alcohol or acetone for 10-30 seconds, rinse with water immediately.
vi. Apply safranin stain for 30 seconds to counterstain Gram-negative bacteria.
vii. After rinsing and drying, observe the slide under an oil immersion lens. Gram-positive bacteria will appear purple, while Gram-negative bacteria will appear red or pink.

5.3.2. Catalase test:

The catalase test is a straightforward biochemical procedure used to identify whether a microorganism produces the enzyme catalase, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. In coconut wine (tuba/toddy) production, this test helps detect catalase-positive microorganisms, which are usually aerobic bacteria or certain yeasts.

Procedure of catalase test for coconut wine:

- **i.** Obtain a sample from the coconut sap or fermentation liquid. Depending on the microorganism being tested, this could be a solid sample (e.g., a colony from an agar plate) or a liquid sample
- **ii.** Place a clean microscope slide on a flat surface. If using a liquid sample, add a small drop to the slide. For bacterial colonies, transfer a small amount from the agar plate directly.
- **iii.** Add a drop of 3% hydrogen peroxide (H_2O_2) onto the sample.
- **iv.** Catalase-positive organisms will immediately release bubbles (oxygen gas) when exposed to hydrogen peroxide. Catalase-negative organisms will not produce any bubbles.
- **v.** Bubbling indicates a positive catalase test, meaning the microorganism produces catalase.
  No bubbling suggests a negative catalase test, indicating the microorganism does not produce catalase.

5.3.3. Amylase test:

The amylase test is used to detect amylase, an enzyme that breaks down starches into simpler sugars like maltose and glucose. In coconut wine production (tuba/toddy), this test is useful because microorganisms that produce amylase, such as certain bacteria and fungi, can aid the fermentation process by converting starches in coconut sap into fermentable sugars for yeast. Amylase-producing organisms like *Aspergillus* or some bacteria play a role in breaking down starches, which improves the availability of sugars for fermentation. This can enhance fermentation efficiency, especially when starch is a major component of the sap. Monitoring amylase activity allows producers to assess microbial involvement and determine whether there is enough sugar for effective fermentation.

Procedure of Amylase test for coconut wine:

- **i.** Collect a sample from coconut sap, fermentation liquid, or from an agar plate with suspected amylase-producing microorganisms.
- **ii.** Use a starch agar plate to test for amylase activity. Inoculate the sample onto the plate.
- **iii.** Incubate the plate for **24-48 hours** at the appropriate temperature
- **iv.** After incubation, flood the plate with iodine solution. Iodine will turn any intact starch blue-black.
- **v.** If the microorganism produces amylase, it will break down starch, creating a clear zone around the colony. If no amylase is produced, the starch will remain intact, and no clear zone will appear.
- **vi.** Clear zone: Positive result, indicating amylase production.
  No clear zone: Negative result, indicating no amylase activity.

5.3.4. Protease test:

The protease test is used to detect protease enzymes that break down proteins into smaller peptides and amino acids. In coconut wine (tuba/toddy) production, this test helps track the role of protease-producing microorganisms, including certain bacteria, fungi, and yeasts, that may be present during fermentation. These microorganisms break down proteins in the coconut sap, influencing the flavour, texture, and overall quality of the final product. Proteases degrade proteins, releasing amino acids that can enhance the wine's flavour profile. Microbial species like *Aspergillus* and *Bacillus*, which produce protease, may assist in fermentation or cause spoilage. Monitoring protease activity helps ensure the quality of the coconut wine by tracking microbial involvement in the fermentation process.

Procedure of protease test for coconut wine:

- **i.** Obtain a sample from coconut sap or fermentation liquid, or use microbial colonies from an agar plate if testing specific microorganisms.
- **ii.** Use a **skim milk agar plate** that contains casein (a milk protein), which will turn clear when broken down by protease enzymes, indicating protease activity.
- **iii.** Place the sample (bacterial, yeast, or fungal culture) onto the skim milk agar plate.
- **iv.** Incubate the plate for **24-48 hours** at the appropriate temperature.
- **v.** After incubation, check for any clear zone around the growth. If the microorganism produces protease, it will degrade the casein, creating a clear area around the colony. If no protease is produced, the casein remains intact, and no clear zone will form.

6. Result and discussion:

Table 1: pH results of different set

Serial no	Set no		pH
1	Set 1A:	Sugar	3.49
	Set 1B:	Date molasses	3.73
	Set 1C:	Sugarcane jaggery	3.84
	Set 1D:	Nalen jaggery	3.60
2	Set 2A:	Sugar	3.49
	Set 2B:	Honey	3.81
	Set 2C:	Sugarcane jaggery	3.84
	Set 2D:	Nalen jaggery	3.60
3	Set 3A:	Malai & Sugar	3.63
	Set 3B:	Malai & Date molasses	3.72
	Set 3C:	Malai & Sugarcane jaggery	3.86
	Set 3D:	Malai & Nalen jaggery	3.63
4	Set 4A:	Malai & Sugar	3.63
	Set 4B:	Malai & Honey	3.80
	Set 4C:	Malai & Sugarcane jaggery	3.86
	Set 4D:	Malai & Nalen jaggery	3.63

Coconut wine should have a pH of three to four. Our produced each set of wine has a pH range between 3 and 4. The table shows the pH levels of various coconut wine samples made using different sweeteners under specific conditions. The experiments are organized into four sets:

- Sets 1 and 2 represent coconut wine prepared without cream (Malai).
- Sets 3 and 4 represent coconut wine prepared with the addition of cream (Malai).

Sugar consistently resulted in the lowest pH value (3.49), indicating it produced the most acidic wine among the sweeteners tested. Sugarcane jaggery recorded the highest pH (up to 3.86), suggesting it generated the least acidic wine. Honey and date molasses produced pH values in the mid-range, with honey exhibiting a slightly higher pH than date molasses. Nalen jaggery showed a moderate level of acidity, with pH values ranging from 3.60 to 3.63. Adding Malai slightly raised the pH in most cases but didn't change the overall acidity trend. For example, sugar-based samples went from 3.49 to 3.63, and sugarcane jaggery from 3.84 to 3.86. This small change suggests Malai may have a mild buffering effect. The sugarcane jaggery samples consistently showed the highest pH in both the plain and Malai-added versions, indicating stable performance across different formulations.

Table 2: Results of Physiochemical analysis

Serial no	Set no		Alcohol concentration	Total phenolic content	Anti oxidant activity	Sugar concentration
1	Set 1A:	Sugar	0.18	1.345	3113.56	0.90
	Set 1B:	Date molasses	0.15	1.709	4222.54	0.91
	Set 1C:	Sugarcane jaggery	0.39	1.765	4788.48	0.89
	Set 1D:	Nalen jaggery	0.16	0.689	3248.25	0.90
2	Set 2A:	Sugar	0.20	1.245	3014.26	0.90
	Set 2B:	Honey	0.32	0.819	2248.87	0.89
	Set 2C:	Sugarcane jaggery	0.38	1.679	4568.80	0.91
	Set 2D:	Nalen jaggery	0.14	0.736	3986.28	0.90
3	Set 3A:	Malai & Sugar	0.63	1.152	3043.14	0.89
	Set 3B:	Malai & Date molasses	0.58	1.682	4830.55	0.92
	Set 3C:	Malai & Sugarcane jaggery	0.54	1.346	4668.76	0.89
	Set 3D:	Malai & Nalen jaggery	0.56	0.672	3668.88	0.90
4	Set 4A:	Malai & Sugar	0.69	1.902	3061.11	0.90
	Set 4B:	Malai & Honey	0.59	0.718	2222.22	0.90
	Set 4C:	Malai & Sugarcane jaggery	0.50	1.721	4663.88	0.90
	Set 4D:	Malai & Nalen jaggery	0.56	0.709	3788.88	0.90

The table presents alcohol concentration levels in different coconut wine samples prepared using various sweeteners, with and without the addition of Malai (cream). The information is organized into four sets:

- Sets 1 and 2 represent coconut wine made without Malai
- Sets 3 and 4 represent coconut wine made with Malai

Among the non-Malai coconut wine samples, sugarcane jaggery produced the highest alcohol content (up to 0.39%), indicating its superior fermentability. Honey yielded moderate alcohol levels (0.32%), outperforming date molasses and Nalen jaggery. Sugar showed low alcohol output (0.18–0.20%), while date molasses and Nalen jaggery had the lowest (0.14–0.16%), possibly due to limited fermentable sugars or fermentation inhibitors. Alcohol levels increased notably in all Malai-added samples, with the highest in Set 4A (Malai & Sugar) at 0.69%, over three times higher than sugar alone. Malai likely boosts fermentation by adding nutrients or fats. Results were consistent across sets. Interestingly, sugarcane jaggery's alcohol yield dropped slightly with Malai, possibly due to interactions affecting fermentation. The table shows how much sugar is left in coconut wine made with different sweeteners, with or without Malai (cream). The sugar levels are mostly between 0.89 and 0.92, with only small changes between samples. Sweeteners like sugar, honey, and date molasses showed consistent sugar concentrations around 0.9% after fermentation. Sugarcane jaggery had the highest value

(0.92% in Set 3C), suggesting higher initial sugar content. Nalen jaggery ranged from 0.89–0.91%, reflecting slight variation. The addition of Malai had minimal impact on sugar concentration, with most samples remaining at 0.9%. However, set 3C (Malai & Sugarcane jaggery) stood out, possibly due to fat interfering with fermentation. Overall, results were consistent across corresponding sets, indicating reliable fermentation and measurement conditions.

The total phenolic content of the wine was calculated using the Folin-Ciocalteau technique. The total phenolic content of coconut wine varied by sweetener and formulation, ranging from 0.672 to 1.902. Phenolic compounds can interact with volatile substances, influencing the release of distinct aromas in wine. The polyphenol content, composition, and antioxidant or radical-scavenging properties of wine are influenced by various intrinsic and extrinsic factors—such as grape variety, cultivation region, climate, fruit quality, and winemaking techniques. These compounds contribute significantly to a wine's antioxidant capacity, as well as its taste, colour, and mouth feel. Sugarcane jaggery showed the highest phenolic content, followed by date molasses, both with and without Malai. Sugar had moderate values, while honey and Nalen jaggery were lower. Malai's effect varied by sweetener, with the highest phenolic boost seen in Set 4A (Malai & Sugar). Malai enhanced phenolic retention in sugarcane jaggery and date molasses but had minimal or negative effects with Nalen jaggery and honey.

Sugarcane jaggery showed the highest antioxidant activity among all sweetening agents, reaching up to 4788.48, followed by date molasses at 4222.54, indicating rich antioxidant content. Sugar and honey showed the lowest activity, with honey at just 2248.87. Nalen jaggery had moderate values. Adding Malai had mixed effects: it increased antioxidant levels when combined with sugarcane jaggery or date molasses but showed no benefit with sugar or honey. For Nalen jaggery, Malai slightly reduced antioxidant activity. Results were consistent across sets, highlighting the reliability of the process and suggesting the potential of specific combinations for enhanced wine quality.

Table 3: Biochemical Analysis

Sample	**Catalase Test**	**Starch Hydrolysis Test**	**Protease Test**	**Gram Staining**
Sugar	Positive	Positive	Positive	Gram-positive bacilli
Sugarcane jaggery	Positive	Positive	Positive	Gram-negative bacilli
Date Molasses	Positive	Positive	Negative	Gram-negative cocci
Honey	positive	Negative	Positive	Gram-positive cocci
Nalen jaggery	Positive	Positive	Positive	Gram-positive bacilli

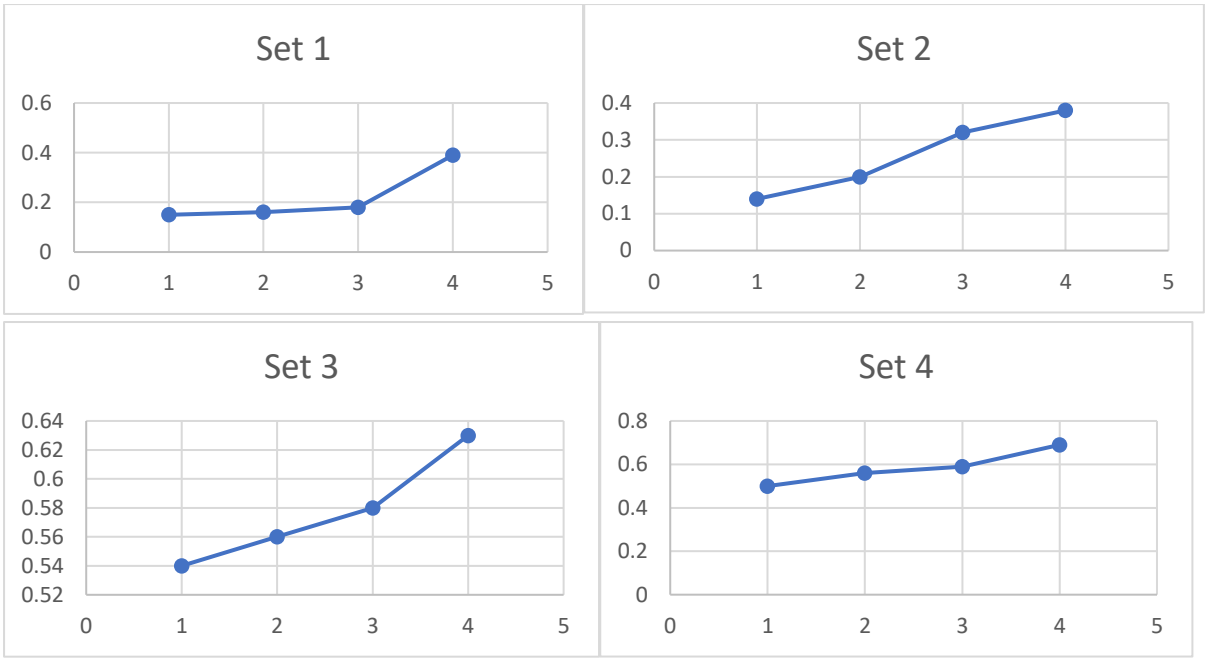


Figure 5: Alcohol Estimation of wines samples

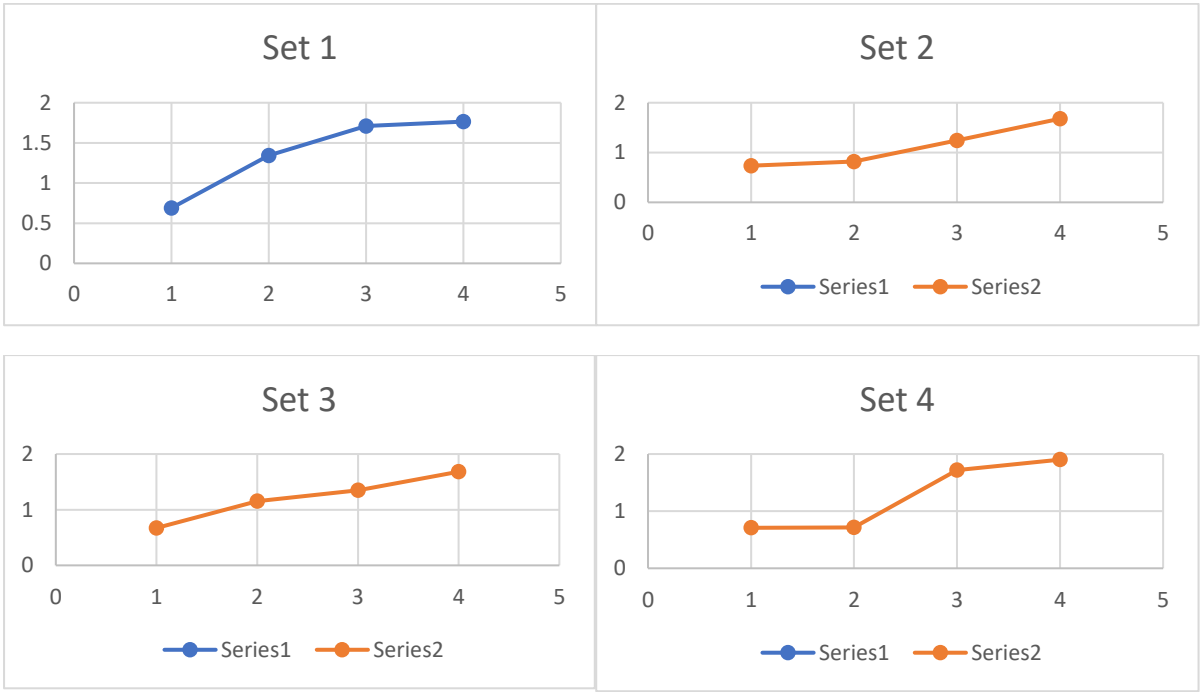


Figure 6: Total Phenolic Content of wine samples

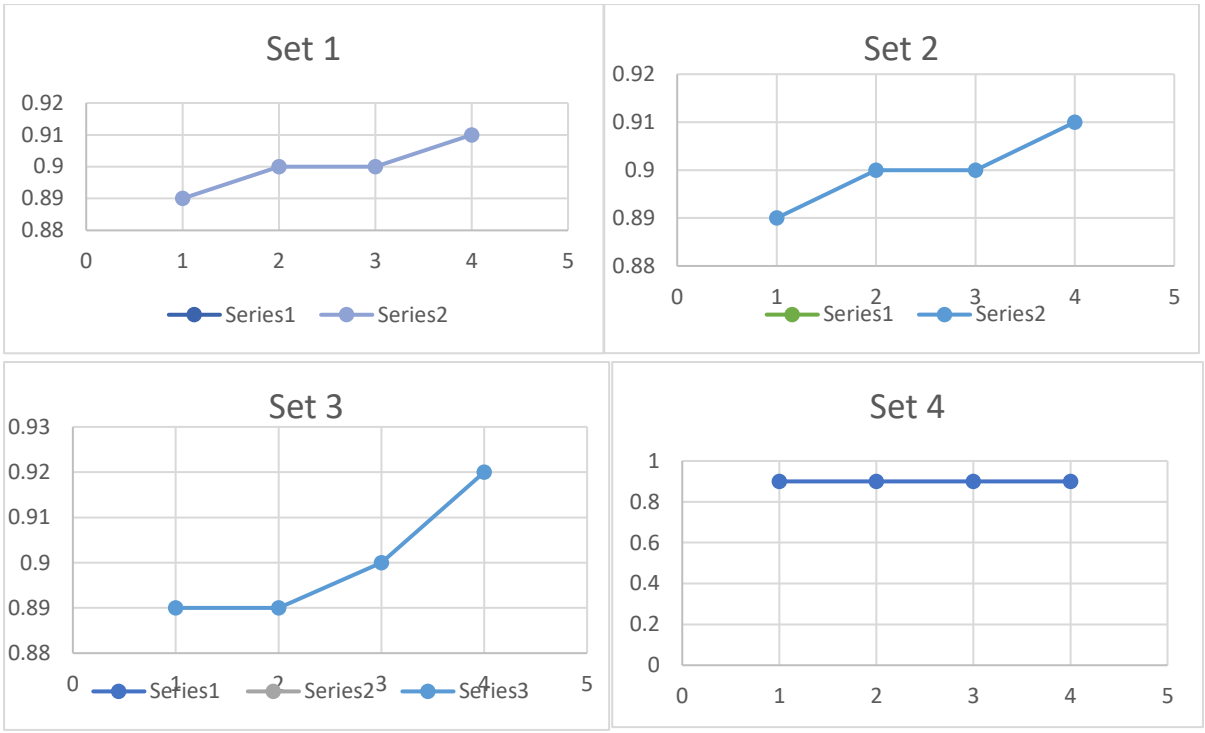
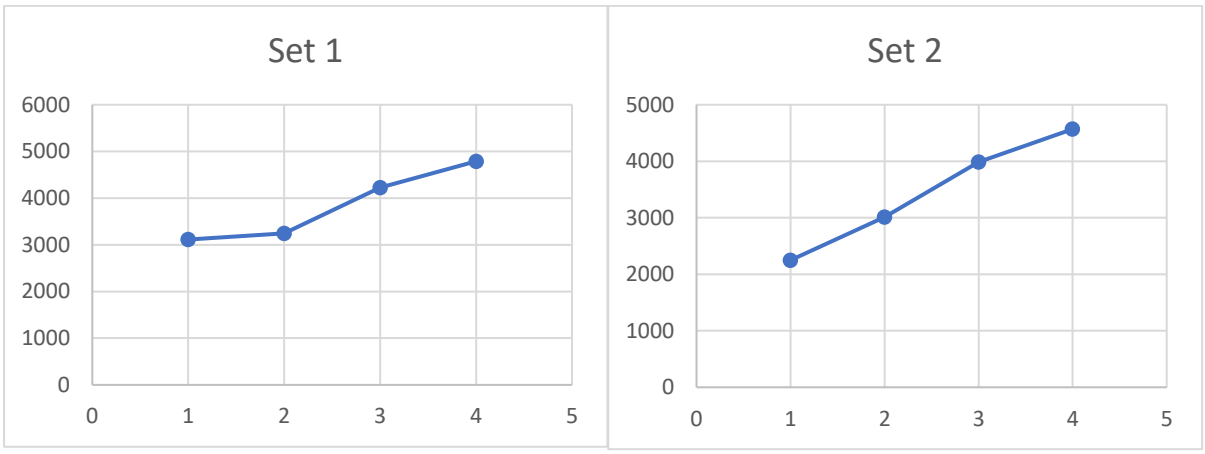


Figure 7: Sugar Concentration of wine samples



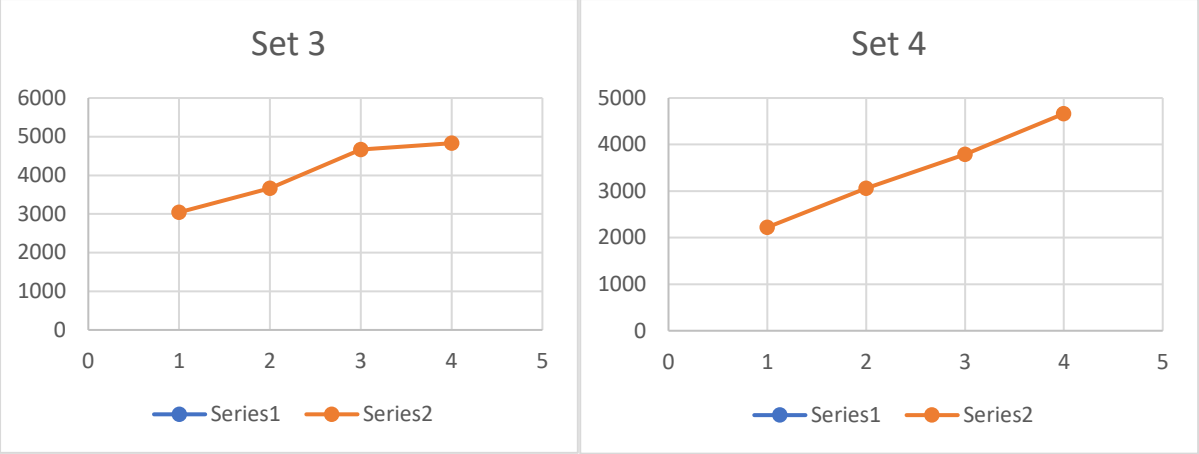


Figure 8: Anti-oxidant Activity of wine samples

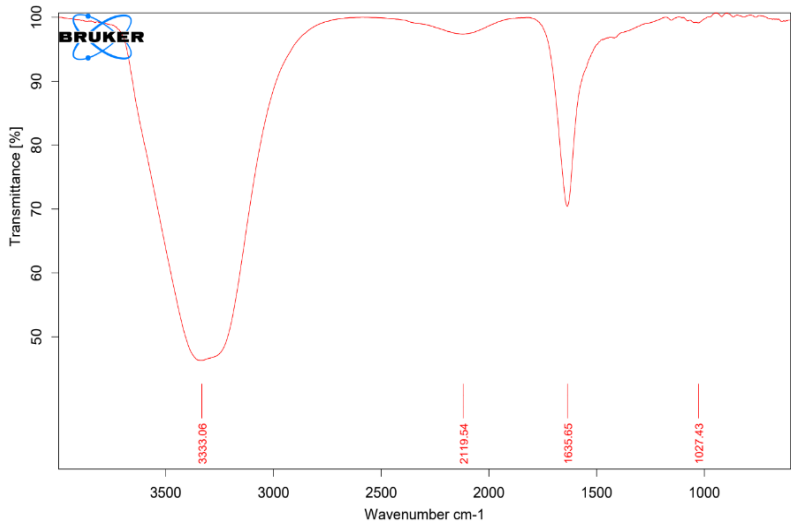


Figure 9: FTIR Spectrum of wine sample

In the FTIR spectrum of wine sample, the first value observed a strong broad and intense absorption peak near 3333.06 cm^{-1} corresponds to the O–H stretching vibrations of hydroxyl groups. This feature reflects the presence of water and extensive hydrogen bonding among hydroxyl-rich compounds, mainly glucose and fructose. The broadness of this peak is indicative of strong hydrogen bonding, which is typical for honey due to its high moisture content and sugar composition. This spectral region is important for evaluating wine content and hydrogen bonding interactions, which directly affect its texture, preservation, and resistance to microbial growth. Therefore, the 3333.06 cm^{-1} peak plays a key role in analyzing

and determining wine quality. The second FTIR peak observed at 2119.54 cm^{-1} in the wine sample is attributed to the C=C=O stretching vibration, indicative of the ketene functional group. Ketenes are reactive unsaturated carbonyl compounds, and although uncommon in wine, they may form through the thermal breakdown of organic acids or esters during fermentation or aging. This distinct, medium-intensity band within the 2100–2150 cm^{-1} range reflects a system with conjugated carbon-carbon and carbon-oxygen double bonds. The others FTIR peak observed at 1635.65 cm^{-1} in a wine sample corresponds to the C=C stretching vibration, typically linked to alkene functional groups. This signal indicates the presence of unsaturated organic molecules, such as polyphenols and natural antioxidants commonly found in wine. These alkenes may originate from grape materials or be formed during fermentation. The peak's clarity and intensity can reflect the amount and chemical behavior of these compounds, which are important for wine's aging properties, colour retention, and antioxidant capacity. The FTIR peak observed at 1027.43 cm^{-1} in the wine sample is attributed to C–F stretching vibrations, indicative of fluoro compounds. This spectral region generally points to alkyl fluorides, which are unusual in natural wine compositions and could indicate contamination from fluorinated cleaning products or packaging sources. The distinct intensity and sharpness of this peak confirm the presence of fluorinated organic compounds. Although such substances are rarely found in wine, their detection is important for ensuring quality control, as they can affect the wine's safety and sensory characteristics.

7. Conclusion:

In the production of coconut wine, this study evaluated the effects of various natural sweetening agents—sugar, sugarcane jaggery, date molasses, Nalen jaggery, and honey—on the quality and biochemical properties of coconut wine, both with and without the addition of Malai (cream). The parameters analyzed included pH, alcohol concentration, total phenolic content, antioxidant activity, sugar concentration, and microbial characteristics.
All wine samples maintained a pH between 3.49 and 3.86, falling within the desirable range (3–4) for coconut wine, ensuring microbial stability and palatability. Among the sweeteners, sugar consistently yielded the lowest pH, indicating a more acidic profile, while sugarcane jaggery produced the least acidic wines with the highest pH values. The inclusion of Malai resulted in a slight increase in pH, suggesting its mild buffering capacity without significantly altering the overall acidity trend. Alcohol concentrations varied notably depending on the sweetener and presence of Malai. Sugarcane jaggery showed the highest alcohol production in non-Malai sets (up to 0.39%), highlighting its superior fermentability. Malai addition boosted alcohol levels significantly across most samples, with Set 4A (Malai & Sugar) recording the highest concentration at 0.69%. This indicates that Malai may enhance fermentation efficiency, likely due to added nutrients or lipids. However, a slight reduction in alcohol yield with sugarcane jaggery after Malai addition suggests a possible interaction inhibiting fermentation. post-fermentation sugar concentrations were relatively stable across all samples, averaging between 0.89 and 0.92%. Sugarcane jaggery in Set 3C showed the highest residual sugar (0.92%), possibly due to unfermented complex sugars or interference from Malai. The consistency of sugar levels across sets indicates reliable fermentation conditions. In terms of Total phenolic content and antioxidant activity were markedly influenced by the type of sweetener used. Sugarcane jaggery led in both parameters, with phenolic values up to 1.765 and antioxidant activity peaking at 4788.48, making it the most effective in enriching wine with health-beneficial compounds. Date molasses followed closely, also offering high antioxidant potential. Sugar and honey displayed moderate to low values. Malai's effect on phenolic retention varied, with the greatest enhancement observed in Set 4A (Malai & Sugar). While Malai positively influenced antioxidant activity in some combinations (notably with sugarcane jaggery and date molasses), it had minimal or even negative effects in others, such as with honey and Nalen jaggery. Biochemical analysis showed diverse microbial communities associated with different sweeteners. All samples tested positive for catalase, indicating active microbial metabolism. Sugar, sugarcane jaggery, and Nalen jaggery supported the presence of protease and amylase activity, essential for fermentation. Notably, sugarcane jaggery and date molasses contained gram-negative microorganisms, while the rest predominantly hosted gram-positive strains. These microbial differences may influence fermentation kinetics and wine flavour profiles. Sugarcane jaggery emerged as the most promising sweetener for coconut wine production due to its high alcohol output, strong antioxidant capacity, and elevated phenolic content. Date molasses also demonstrated beneficial properties, particularly in enhancing antioxidant activity. Sugar, while less effective in fermentation, showed improved characteristics when combined with Malai. Honey and Nalen jaggery produced acceptable results but were less favourable in terms of antioxidant activity and fermentability. The addition

of Malai positively influenced alcohol production and, in some cases, enhanced phenolic retention, though its impact varied depending on the sweetener. These findings underscore the importance of selecting appropriate sweetener and formulation combinations to optimize the quality, nutritional value, and sensory properties of coconut wine. In conclusion, coconut wine quality can be significantly modulated by the choice of sweetener and the inclusion of Malai. Sugarcane jaggery, particularly in non-Malai formulations, offers the best overall profile, suggesting its suitability for high-quality coconut wine production. Further studies can explore flavor profiling, sensory analysis, and storage stability to support commercial viability.

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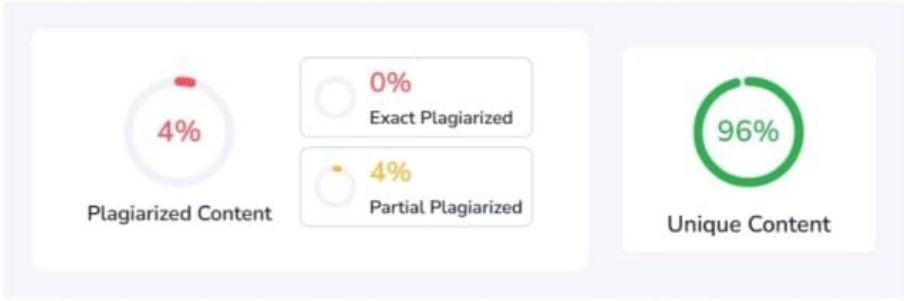

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ABSTRACT

This study focused on the development and detailed evaluation of coconut wine fermented using Saccharomyces cerevisiae, examining the effects of different sweeteners—sugar, date molasses, sugarcane jaggery, honey, and Nalen jaggery—as well as the addition of coconut pulp (Malai). Four groups of coconut wine formulations, with and without Malai and incorporating the various sweeteners, were fermented over 48 hours. The resulting wines were thoroughly analyzed for several physicochemical properties, including pH, alcohol content, residual reducing sugars, total phenolic content (TPC), and antioxidant activity measured by the DPPH assay. Additionally, Fourier Transform Infrared Spectroscopy (FTIR) was used to detect chemical bond characteristics, and biochemical tests were carried out to evaluate the metabolic traits of the microbial strains involved. All wine samples exhibited pH values within the favourable range of 3.49 to 3.86. Among the sweeteners tested, sugarcane jaggery supported the highest levels of alcohol production—0.39% without Malai and up to 0.50–0.54% when Malai was included. In contrast, plain sugar resulted in the lowest alcohol yields. Generally, the presence of Malai improved alcohol output across most formulations. Wines made with sugarcane jaggery also showed the highest total phenolic content and antioxidant activity, suggesting potential health benefits. Biochemical tests of bacterial isolates revealed a range of enzyme activities (catalase, amylase, protease) and varied Gram staining patterns influenced by the type of carbon source used. FTIR analysis enabled molecular-level distinctions among different wine samples, variations influenced by the type of sweetener used and the inclusion of Malai. In summary, the type of sweetener used plays a critical role in influencing the fermentation efficiency and the functional quality of coconut wine. Sugarcane jaggery stood out as the most effective sweetener, enhancing both ethanol production and antioxidant capacity. The addition of Malai further supported alcohol fermentation, likely by offering supplementary nutrients or creating favourable conditions for yeast metabolism. These findings offer important guidance for optimizing coconut wine formulations to achieve specific quality and functional attributes.

Keywords: Coconut wine, Cocos nucifera, Saccharomyces cerevisiae, FTIR spectroscopy, pH, Total phenolic content (TPC), Antioxidant activity (DPPH assay), Alcohol concentration, Alcoholic beverage

INTRODUCTION

Wine is a classic fermented alcoholic beverage created using yeast.Wines are undistilled drinks generally produced from fruit juices that are nutritious, mildly stimulating, and more flavourful than standard fruit juices. These fruits undergo fermentation, during which microbial processes play a crucial role in the final product's quality. Generally, wine contains an alcohol attention between 5 to 13 percentages.Wine possesses a flavour reminiscent of fresh fruit and can be stored and transported under conventional conditions. As an undistilled product, it retains most of the nutrients found in the original fruit juice. A standard wine comprises ethyl alcohol, sugar, acids, higher alcohols, tannins,

aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, and trace compounds that contribute to its flavour (Sreelekshmi et al. 2018). Fermented beverages and alcoholic drinks are culturally and socially embraced for various purposes, including consumption, entertainment, traditional customs, and religious ceremonies (Ezemba and Archibong 2017). Winemaking techniques are popular globally, spanning Europe, America, Australia, Asia, and recently Africa. For millennia, wine has served as a traditional medicinal drink and features prominently in various religious narratives. The production of wine is achieved primarily through anaerobic fermentation. Wine is believed to contribute to health, not only due to its nutritional benefits but also because it can substitute for unsafe, poor-quality, or unsatisfactory water supplies. It contains a significant amount of dietary fibre, which is helpful for individuals dealing with constipation. Additionally, it supports muscle growth in those who are thin or underweight (Ezemba and Archibong 2017).

The coconut, a fruit from the coconut win (Cocos nucifera), is piece of the Arecaceae kindred. It thrives in tropical areas typically located between 22° north and south of the equator, primarily along coastlines (Cuarto and Magsino 2017). The coconut palm is distributed across the western tropical lowlands but is capable of growing in any location with ample rainfall and warmth. While its exact origins remain unknown, it has been utilized for thousands of years, with Malaysia or Indonesia being the most probable early homes. One certainty is that humans have played a significant role in its dissemination throughout tropical regions, making it a common aspect of these areas (Bisht and Singh 2024).

Figure 1:Coconut wine (Tuba)

The term "coconut" can refer to the entire coconut palm, its seed, or the fruit itself. Botanically, it is considered a drupe rather than a true nut. Similar to other fruits, it consists of three layers: the exocarp, mesocarp, and endocarp. Inside the hard shell lies the seed, and when it germinates, the root of its embryo emerges through one of the shell's three "eyes" (Beveridge et al. 2022). Coconut water is the clear liquid present inside coconuts (the fruit of the coconut palm). This coconut water (also known as coconut liquid endosperm) is recognized as one of the most versatile natural products in the world. It occurs naturally, is extremely high in potassium, contains sodium chloride and carbohydrates, and is regarded as the preferred hydrating beverage in some regions In medical environments, coconut water can be utilized as an oral rehydration solution to restore fluids lost due to gastrointestinal issues, particularly in patients suffering from severe dehydration caused by diarrhoea. Meanwhile, an alcoholic beverage is characterized as a consumable liquid containing alcohol. There are three main categories of alcoholic beverages: fermented drinks, distilled spirits, and fortified wines. Fermented beverages are made from fruit juice or plant sap through natural fermentation processes. In India, various palms, including wild dates (Phoenix sylvestris), coconut palm (Cocos nucifera), and Palmyra (Borassus flabellifer), are commonly utilized for wine production. Wine is a type of alcoholic drink produced through the fermentation process. The yeast used in winemaking consumes sugar, converting it into ethanol, carbon dioxide, and heat (Zulaikhah 2019).

Coconut wine is a light pale beverage with a strong aroma and a sweet, refreshing flavour. It is a fermented drink produced from coconut water through the process of fermentation with the help of yeast (Jagadeesh et al. 2019). /.

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The coconut, a fruit from the coconut win (Cocos nucifera), is piece of the Arecaceae kindred.

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A standard wine comprises ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, and trace compounds that contribute to its flavour (Sreelekshmi et al. 2018).

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